Dynamic Tracking of Osteoblastic Cell Traction Force during Guided Migration

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Abstract—Introduction—Continuous development of cell traction force can regulate cell migration on various extracellular matrixes *in vivo*. However, the topographical effect on traction force is still not fully understood.

Methods—Micropost sensors with parallel guiding gratings were fabricated in polydimethylsiloxane to track the cell traction force during topographical guidance in real time. The force distributions along MC3T3-E1 mouse osteoblasts were captured every minute. The traction force in the leading, middle, and trailing regions was monitored during forward and reversed cell migration.

Results—The traction force showed periodic changes during cell migration when the cell changed from elongated to contracted shape. For cell migration without guiding pattern, the leading region showed the largest traction force among the three regions, typically 5.8 ± 0.8 nanonewton (nN) when the cell contracted and 7.1 ± 0.5 nN when it elongated. During guided cell migration, a lower traction force was obtained. When a cell contracted, the trailing traction force was 4.1 ± 0.4 for non-guided migration and 2.2 ± 0.2 nN for guided migration. As a cell became elongated, the trailing traction force was 6.0 ± 0.5 nN during non-guided migration and 4.8 ± 0.3 nN under guidance. When a cell reversed its migration direction, the magnitudes of the traction force from the leading to the trailing regions also flipped.

Conclusion—The cell traction force is continuously influenced by topographical guidance, which determines cell migration speed and direction. These results of cell traction force development on various topographies could lead to better cell migration control using topotaxis.

Keywords—Cell traction force, Real-time tracking, Cell speed, Topographical guidance, Forward and reversed.

INTRODUCTION

Cell migration is responsible for various physiological events, such as embryonic development,³⁷

wound healing,¹⁰ and cancer metastasis.⁶ For example, bone formation is initiated by migration and recruitment of osteoblasts, followed by proliferation, differentiation, and matrix generation to form solid bone tissue.⁴³ Therefore, understanding osteoblastic cell migration is essential for bone tissue engineering. It is known that not only the chemoattractants,^{21,33} but also the extracellular physical properties can affect osteoblastic cell migration.²² As osteoblasts originate from the differentiation of osteogenic cells in the periosteum and in the endosteum, the osteoblastic cell migration is affected by the physical properties of the localized extracellular matrix (ECM). The periosteum tissue consists of fibrous layers that are well-organized in the direction of bone growth,²⁸ hence the osteoblastic cell migration is regulated by topographies similar to parallel gratings. The interaction between cells and topographies is known as contact guidance, which can influence cellular migration behaviours *via* integrin-ECM adhesion, actin polymerization, and nucleus deformation.^{34,42}

As a cell makes contact with the ECM, traction force is developed. Through the bonding of the transmembrane adhesion molecules to the ECM ligands such as fibronectin (FN), the linkage between the ECM and the cytoplasmic is built through the formation of focal adhesions (FAs) with cytoskeletal elements called microfilaments.³³ The localized traction force is exerted on the ECM *via* the adhesive sites and it could affect the formation of cytoskeleton and the assembly of matrix adhesion.^{25,26} The physical properties of the ECM, such as surface topography and stiffness gradient, can influence the cell traction force during migration.^{31,39,42} Hence cell migration, including migration direction and speed, can be controlled by the physical conditions of the contact surface.³⁰ Measuring the cell traction force during migration will provide important information related to the interactions

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between osteoblastic cells and ECM, which will be useful for designing guiding patterns to control cell migration in tissue.

The advancement of micro- and nano-technology has created the possibility of developing microsystems that can be used to study cell traction force. Over the past decades, various microfabricated platforms have been used, such as optical tweezers integrated with magnetic beads,⁴⁰ silicon (Si) and polymeric cantilever sensors,¹⁸ micro-strain piezoelectric force sensor,¹² silicone rubber substrate wrinkling,²³ and traction force microscopy.^{1,8,32} Although these techniques provided effective methods to investigate the mechanical force at the cellular level, there are some drawbacks such as the lack of resolution to map the traction force distribution,^{12,18} the inability to track cellular force distribution dynamically,²³ and no topographical guidance for cell migration.^{1,8,32} To better understand the mechanisms during cell migration under contact guidance, micropost arrays were integrated with parallel gratings to measure the osteoblastic cell traction force under topotaxis. The micropost sensors, with typical size of few micrometers (μm), can be densely positioned next to the guiding patterns to measure cellular traction force with high resolution. As cells move on top of the microposts, each micropost will bend as a linear elastic beam under shear force. By adjusting the micropost dimensions such as diameter (dia.) and height, the sensitivity of the elastic sensors can be optimized.¹⁷ Hence elastic post sensors have the advantages that they can be integrated with topographical patterns for guided cell migration and they provide high sensitivity to map localized traction force with high resolution.^{38,45,46}

Previous reports of using micropost arrays as sensors mainly showed static cell traction force without tracking dynamic force distribution over time.^{7,13,29,35,41} For example, micropost sensors were used to study cellular force-dependent regulation of focal adhesion.⁴¹ Other studies have investigated cell-cell interaction,²⁹ cellular sheet interaction,¹³ and transmembrane migration.³⁵ However, the cellular mechanics during directional migration are expected to change dynamically in a cyclic manner,^{20,25,27} during which the cell traction force distribution from the leading to trailing regions is known to affect the cell migration process.⁴⁴ Very little is known about how the traction force distribution is related to cell migration direction and speed. The real time measurement of traction force from the leading to trailing regions in a cell during migration could provide insights in cell migration dynamics. In addition, although the effects of durotaxis³ and chemotaxis³⁶ on cell traction force have been investigated previously, the influence of topotaxis on traction force is still unknown. Therefore, it is essential to investigate how topographical patterns could affect the distributions of traction

force in osteoblasts during guided, directional migrations. This information will provide the design guidelines for controlling cell migration in ECM, which could be applied to cell screening or tissue regeneration.

METHODS

Fabrication of Micropost Sensing Arrays

SU-8 (Microchem, MA, USA) master molds containing guiding and sensing patterns were fabricated using ultra-violet (UV) photolithography. A layer of SU-8 2005 polymer with a thickness of $4.8\ \mu\text{m}$ was spin coated onto a Si wafer and flood exposed using 320 nm (nm) UV light at an intensity of $10\ \text{mW}/\text{cm}^2$ for 30 s. This would fully cross-link the SU-8 and work as an adhesive layer to promote the adhesion between the subsequent thick SU-8 layer and the Si wafer. A second SU-8 2010 polymer was spun onto the adhesive layer, as shown in Fig. 1a. After a 10 s UV exposure and development, the patterned SU-8 mold was created, as shown in Figs. 1b and 1c. The typical size of a sensing post array is $3\ \mu\text{m}$ in dia. and $4\ \mu\text{m}$ in spacing, whereas the guiding pattern has $5\ \mu\text{m}$ wide lines and $5\ \mu\text{m}$ wide spaces. The patterned SU-8 mold was coated with trichloro (1H,1H,2H,2H-perfluorooctyl) silane (FOTS) (Sigma-Aldrich, WI, USA) as an anti-sticking layer to allow easy demolding. The coating was performed by vaporizing $50\ \mu\text{L}$ FOTS at 80°C for 90 min. Polydimethylsiloxane (PDMS) prepolymer (PDMS base monomer:curing agent weight ratio = 10:1, Sylgard 184, Dow Corning, MI, USA) was poured onto the SU-8 mold and degased in a vacuum chamber at 10^{-2} mbar for 45 min, followed by curing at 110°C in an oven for 15 min, as shown in Fig. 1d. After demolding the PDMS from the SU-8 mold, it was silanized for the second replication. A second layer of PDMS prepolymer was spin coated onto the soft PDMS mold to reach a uniform thickness, as shown in Fig. 1e. After degasing for 20 min at 10^{-2} mbar in the vacuum chamber, the PDMS soft mold along with the second PDMS prepolymer on the top was covered with a glass slip and cured in a 110°C convection oven for 6 h to obtain fully cured PDMS. The patterned PDMS platform with the same polarity as the SU-8 mold was generated by peeling off the soft PDMS mold, as shown in Fig. 1f. To separate the collapsed PDMS sensing microposts, the platform was ultrasonicated in absolute ethanol ($\geq 99.8\%$, Sigma-Aldrich, WI, USA) and supercritically dried with a critical point dryer (EM CPD300, Leica, Hesse, Germany). The patterned PDMS platform on glass was then mounted onto a 35 mm confocal dish with a 20 mm opening for time lapse imaging.

FIGURE 1. Schematic of fabrication process for patterned PDMS platform. (a)–(c) Ultra-violet (UV) lithography using SU-8 photoresist enables creation of hard mold with high aspect ratio. (d)–(f) Double cast PDMS to generate sensing and guiding patterns. (g), (h) Functionalize surface of PDMS with O_2 plasma, coat top of PDMS patterns with FN to promote cell adhesion and cover sidewall with Pluronic F-127. (i) Micrograph of fabricated PDMS platform. Cell guiding patterns consist of parallel gratings, which are $5.7\ \mu\text{m}$ in width and $4.3\ \mu\text{m}$ in spacing. Micropost sensing arrays, which are $3\ \mu\text{m}$ in dia. and $4\ \mu\text{m}$ in spacing, are integrated in between guiding gratings. (j) Micrograph of PDMS sensing posts arrays. Each post is $3\ \mu\text{m}$ in dia. and $13.4\ \mu\text{m}$ in height.

Surface Functionalization of Sensing Platforms

Contact printing was used to transfer ECM protein onto the top of the PDMS platform to enable cell attachment and spreading. First, the PDMS base and curing agent were mixed at a weight ratio of 20:1, degassed at 10^{-2} mbar vacuum chamber for 20 min, and cured in $110\ ^\circ\text{C}$ convection oven for 10 min to generate flat $1 \times 1 \times 0.5\ \text{cm}^3$ PDMS pads. The PDMS pads were coated with $40\ \mu\text{L}$ bovine FN ($50\ \mu\text{g}/\text{mL}$, Sigma-Aldrich, MO, USA) for 3 h at $4\ ^\circ\text{C}$. The excess FN was rinsed with deionized (DI) water. The patterned PDMS-sensing platform was hydrophilized by an O_2 plasma using a reactive ion etching system (790 RIE system, Plasma-Therm, FL, USA) with 10 sccm O_2 , 80 mTorr, and 30 W rf power for 30 s. The FN-coated PDMS pad was placed in contact with the PDMS-sensing platform for 30 s, and the FN from the pad was transferred to the top of the PDMS sensing posts and guiding patterns, as shown in Fig. 1g. The FN-coated PDMS platform was stained with a lipophilic dye (DiI, 1,10-dioleil-3,3,30,30-tetramethylindocarbocyanine methanesulfonate, Invitrogen, CA, USA) at $5\ \mu\text{g}/\text{mL}$ and incubated for 1 h at $4\ ^\circ\text{C}$. This dye helped to enhance the contrast for the displacement measurement during traction force analysis. After thorough rinsing with DI water thrice to remove excess DiI, the PDMS platform was immersed in 0.2% Pluronic F-127 (Sigma-Aldrich, WI, USA) for 30 min at $4\ ^\circ\text{C}$, as shown in Fig. 1h. The immersion in Pluronic solution can prevent further absorption of FN to the sidewalls of microposts and thus prevent cell adhesions to areas in between the posts. Finally, the patterned PDMS platform was rinsed with DI water and submerged in phosphate buffered saline (PBS) for cell culture and confocal imaging.

Cell Culture on PDMS Platform

The MC3T3-E1 osteoblastic cells (ATCC numbers CRL-2594) were maintained in Dulbecco's modified eagle medium (DMEM) at $37\ ^\circ\text{C}$ in 5% CO_2 in a humidified incubator. DMEM consisted of high glucose (Invitrogen, CA, USA) with 10% fetal bovine serum (FBS) (Gibco, MD, USA), antibiotic–antimycotic (100 units/mL of penicillin, 100 mg/mL of streptomycin, and 0.25 mg/mL of Amphotericin B, Gibco, MD, USA), and 2 mm alanyl-L-glutamine (Gibco, MD, USA). The medium was refreshed every 3 days and the cells were kept below full confluence at all times. Before seeding the cells, the patterned PDMS platform was sterilized in 95% ethanol for 15 min and washed with PBS four times. The MC3T3-E1 cells were seeded at a density of $7 \times 10^3\ \text{cells}/\text{cm}^2$. The

culture dish was preserved at 37 °C in 5% CO₂ in a humidified incubator for 6 h, and the cells were allowed to attach and spread on the PDMS platform. After cell spreading on the platform, the medium was replaced by a CO₂ independent medium (Invitrogen 18045–088, CA, USA), 10% FBS, and antibiotic–antimycotic, and supplemented with 2 mM alanyl-L-glutamine (Gibco, MD, USA).

Time-Lapse Imaging

Time-lapse images were captured using a laser scanning confocal microscope (TCS SP5 with 543 nm HeNe visible laser, Leica, Hesse, Germany) at a time interval of 1 min for 15 h. The images were taken at four adjacent positions to observe the cell traction force of multiple cells. A 40 × oil immersion objective lens was used for imaging the cells under high magnification. To keep images in focus, the incubator was preheated at 37 °C for 2 h before imaging and stage movement was controlled within 1 mm. To acquire the bending of PDMS posts, images at the bottom and on the top of the microposts were captured under the confocal microscope. Images at the bottom represented the original positions of the microposts, whereas images on the top showed the micropost bending due to the cell traction force. For accurate analysis of the cellular shape and micropost position, time-lapse images in bright field and fluorescent images of the stained platform were captured. Photons at 543 nm were used to excite the DiI and its fluorescence signal was detected at 570 nm.

Analysis of Bending Posts

Image analysis was performed on the grey-scale images obtained from the confocal time-lapse with an interval of 1 min. A custom-programmed MATLAB (R2007b, The MathWorks, MA, USA) graphical user interface (GUI) was used to process the images to determine the post displacement to quantify the cell traction force. First, a batch of three images, including a bright field image for cell recognition, a fluorescent image focused at the top of the post, and a fluorescent image focused at the base of the post were loaded into the GUI. To obtain the displacement of the bent microposts due to cell traction force, a shape-fitting algorithm was used to determine the central positions at the top and bottom of the posts. The traction force was calculated using the multiplication of the displacement with the spring constant, which was 8.84 nanonewton/μm (nN/μm), and will be discussed in detail in the modeling section. The free microposts, which did not come in contact with cells, were analyzed to determine the mean displacement error. The analysis showed a mean displacement error of 0.03 μm,

which corresponded to the mean force error of 0.27 nN. The cell movement was tracked by the shift of the cell center using NIH ImageJ software (version 1.48v). All quantitative results in this study were reported as mean ± standard error. Statistical significance was tested by employing Student's *t* test, where null hypothesis was rejected at $p < 0.05$.

RESULTS

Design of Cell Guiding Patterns and Modeling of PDMS Micropost Sensors

The cell traction force was evaluated during migration on the PDMS platform with and without integration of guiding patterns. Cells typically move randomly in all directions unless they are guided by asymmetrical patterns.⁴² A typical guiding pattern is shown in Fig. 1i, which consisted of five rows of parallel gratings with 5.7 μm wide ridges and 4.3 μm gaps in between five rows of micropost sensing arrays. The microposts, which were designed to be 3 μm in dia. and 4 μm in spacing, could be used to map the dynamic changes of the cell traction force during migration.

Cell traction force is generated through the movement of adenosine triphosphate-dependent motor proteins, which are called myosin, to produce pulling force acting on the actin filaments on the cytoskeleton.⁴ As a cell develops FAs through the interactions between integrin receptors localized on the cell membrane and the ECM on the platform, the linkage between cytoskeleton–integrin and ECM builds up and the microposts would be deflected. Despite the discrete topography of the microposts compared with that of a flat surface, cells show similar spreading and migration behavior.⁴⁶ The top surface of the microposts was selectively coated with FN to promote the development of FAs, whereas the Pluronic coating on the sidewalls of the microposts helped prevent cell trapping in between the microposts. A computational analysis was performed to calculate the deflection of these PDMS microposts when cells exerted traction force on top of microposts using the commercially available finite element methods (FEM) suite (COMSOL Multi-physics 4.3b, COMSOL, Inc., MA, USA). The FEM model consisted of a cylindrical PDMS post with the dimensions corresponding to the posts shown in Fig. 1j. The dia. and the height of the microposts were 3 and 13.4 μm, respectively. PDMS cylinder was considered a Neo-Hookean hyperelastic model having a uniform elastic modulus distribution of 2.5 megapascal.⁴⁶ Tetrahedral mesh elements with a minimum size of 0.24 μm were used to discretize the assembly. As depicted in Fig. 2a, the bending of a micropost was calculated under a horizontal traction force of 20 nN

FIGURE 2. (a) Graphical depiction of finite element method analysis of elastic post sensor with dia. of $3 \mu\text{m}$ and height of $13.4 \mu\text{m}$. Bending of post is in response to horizontal traction force of 20 nN applied on top surface of PDMS post. Colour chart indicates horizontal displacement under 20 nN traction force. (b) Displacement of centre of post top as function of applied traction force on post top. Nominal spring constant of PDMS posts can be derived from slope of displacement curve.

exerted uniformly on top. At 20 nN traction force, the typical displacement on top of the micropost was found to be $2.25 \mu\text{m}$. The nominal spring constant (k_{nom}) of a micropost could be determined as:

$$k_{\text{nom}} = \lim_{F \rightarrow 0} dF/dx \quad (1)$$

where F is the force applied on top of the post and x is the corresponding displacement. The spring constant was quantified as $8.84 \text{ nN}/\mu\text{m}$ by linearly extrapolating the traction force to zero and applying (1), as shown in Fig. 2b. The traction force during mesenchymal migration was in the range of $3\text{--}40 \text{ nN}$,^{17,18} in which the microposts were linearly deformed.

Dynamic Changes in Traction Force and Cell Morphology During Directional Migration

Figure 3 shows the changes in both the cell morphology and traction force for an MC3T3-E1 cell during forward migration, in which the cyclic behavior was similar to the previously reported directional cell

FIGURE 3. Force mappings during directional cell migration without guiding pattern. (a) Cell started migration in contracted shape. (b), (c) Cell elongated along migration direction and exerted increasing traction force in both leading and trailing regions. (d) Trailing adhesive sites released and cell shape became contracted, which started second migration cycle. Contour of cell is indicated by yellow dashed line. Starting and ending positions are indicated by asterisk and dot in micrographs, respectively. Traction force is indicated by white arrow. Cell migration direction is described as shift of centroid of cell from beginning to end during single migration cycle, which is labelled using blue arrow.

migration.^{16,27} A typical time-lapse movie of a cell moving on top of the sensing posts is shown in movie S1 in the Supporting Materials. The MC3T3-E1 cell showed a contracted shape when directional migration started, as shown in Fig. 3a. The traction force was small across the whole cell. The traction force in the leading region was directional and pointed towards the cell center, whereas the force in the trailing and middle regions was randomly distributed. During the migration, both the leading and trailing regions protruded, as shown in Fig. 3b. As the cell extended the lamellipodia in both regions and became polarized, the measured traction force increased gradually and pointed towards the cell center near the leading and trailing regions but remained low in the middle of the cell. This result could be related to the development of focal adhesion complexes, and thus myosin-based contraction could introduce increased deformation of the microposts near the leading and trailing regions.²⁰ The cell traction force continued to increase in the leading and trailing regions, but the cell did not show much movement, as shown in Fig. 3c. Subsequently, the traction force dropped in both the leading and trailing regions after the release of adhesive sites at the trailing end, as shown in Fig. 3d.

The trailing region retracted and the cell moved forward and contracted again. This cyclic behavior would repeat itself as the cell continued the forward migration.

The collective force from all positions within a cell is related to cell migration.^{9,20,30,37} Cell traction force is expected to vary from the leading to trailing regions.² Therefore, a cell is divided into three different regions of equal length along its long axis. These regions are the leading, middle, and trailing regions. The resultant force was obtained by adding all the force vectors sensed by the microposts covered by a given cell. A cell would continuously change its shape during migration and these three regions were adjusted accordingly. Moreover, to correlate the net traction force with the migration direction, the angle (θ) of the resultant traction force in each region was compared with the direction of migration, as shown in Fig. 4a. The average cell traction stress was also analyzed to show the stress distribution from the leading to the trailing regions, as shown in figure S1. The traction stress was found to be higher near the two ends of the cell, namely 0.25 ± 0.01 nN/ μm^2 near the leading region and 0.22 ± 0.01 nN/ μm^2 near the trailing region. These traction stress measurements were in agreement with the previously reported results for 3T3 fibroblast cells.^{2,9,41} The cell traction stress was much higher near the two edges compared to the center of the cell.

When cells were in contracted shape, the cell traction force direction, θ , showed larger deviations from the migration direction compared to when cells were in elongated shape as shown in figure S2. For contracted cells, 43.0% of the cells in the leading region had θ within 15° , whereas 27.8 and 12.7% of cells showed θ within 15° in the trailing and middle regions, respectively. As cells became elongated, the cell traction force directions in the leading and trailing regions were in closer alignment with the migration direction, as shown in Fig. 4b. The traction force in the leading and trailing regions had opposite directions and they both pointed towards the center of the cell, similar to previously reported results.⁴⁶ In Fig. 4b, 65.8% of the cells in the leading region had θ within 15° of the migration direction, whereas 38.7 and 14.4% of cells showed θ within 15° in the trailing and middle regions, respectively.

The numbers of microposts covered in the leading, middle, and trailing regions were different, because MC3T3-E1 cells showed asymmetrical cytoplasmic spreading between leading and trailing regions during directional migration.⁴² To account for these variations, the traction force from the leading to trailing regions was normalized by the number of covered microposts. Normalized cell traction force in the three regions and the speed of cell migration were analyzed as a function of time. As illustrated in Fig. 4c, cells had contracted shape at 0 min and the cellular force in

FIGURE 4. (a) Leading, middle, and trailing regions along cell major axis. Traction force is analyzed by adding all force vectors in each of three regions. Angles θ indicates directions of traction force in each region by comparing resultant traction force with migration direction. Cell contour is indicated by yellow dashed line. Traction force is indicated by white arrows. Cell migration direction is labelled using blue arrow. (b) Force direction θ relative to migration direction in leading, middle, and trailing regions with total 41 cells analyzed. (c) Normalized cell traction force and speed as function of time for MC3T3 cell underwent forward migration on micropost arrays without guiding pattern. Arrows indicate moments when trailing region was released, starting at 75 and 117 min.

both the leading and trailing regions was relatively small. The traction force increased up to 75 min as the cells elongated and became polarized. As cells elongated, the traction force in the leading region developed to be larger than the trailing region. As shown in Figs. 3c and 4c, the traction force in both the leading and trailing regions dropped when the trailing region started to release. After the complete release of the trailing region at 81 min, the cell contracted and the next migration cycle started as the cell traction force and shape changed in a similar cyclic manner.

FIGURE 5. (a) Normalized cell traction force in leading, middle, and trailing regions while cell shape contracted or elongated. (b) Average number of covered microposts in leading, middle, and trailing regions while cell shape contracted or elongated. Results are reported as mean $\pm$ SE and 23 cells were analyzed. Statistical significance was calculated using Student's t-test (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

As shown in Fig. 4c, the cell speed changed during migration. The cell speed at the beginning of the migration cycle was low as cells started to elongate with protruding lamellipodia. When the traction force increased as the cell elongated, the cell hardly moved. The cell speed increased when the trailing region released and the posterior shifted towards the leading region. The cell speed dropped after the cells became contracted again and the next cell migration cycle started.

The normalized traction force in the leading, middle, and trailing regions during cell elongation and contraction was shown in Fig. 5a. The normalized traction force in the leading region, 5.8 ± 0.8 nN, was higher than the 4.1 ± 0.4 nN in the trailing region when a cell started migration in its contracted shape. After the cell became elongated, the traction force increased to 7.1 ± 0.5 nN in the leading region and 6.0 ± 0.5 nN in the trailing region. The traction force in the leading region continued to be larger than the trailing region. The traction force in the middle region of a cell remained constantly small among three regions and independent of the cell shape.

The average numbers of covered microposts in each region during contraction and elongation are shown in Fig. 5b. When cells were contracted at the beginning of the migration cycle, the numbers of covered microposts

in the leading and trailing region were similar. The cells spread out over a larger area and covered more microposts in the leading region during elongation compared with during contraction.

The net traction force in the leading, middle, and trailing regions without normalization was shown in figure S3. The trends of total resultant force development in the leading, middle, and trailing regions were similar to the normalized traction force shown in Fig. 4c. As the leading region tended to spread and contact more microposts while the cell elongated, the total traction force in the leading region increased as the cell shape changed from contracted to elongated. When a cell was in a contracted shape, the cell traction force direction during migration was random, as shown in Figs. 3a and S2. When the cell became elongated, the traction force in the leading and trailing regions increased and pointed towards the cell center, the net force vector cancelled out each other as shown in Fig. 3b. As shown in figure S4, the cell area and aspect ratio were fluctuating as the cell stayed elongated with very low speed. The cell traction force in the leading and trailing regions continued to build up to maximum values before the trailing region was released, as shown in Fig. 3c. After the release of the trailing region, cell traction force decreased as shown in Fig. 4c, while the cell speed became higher and the cell area decreased as the cell became contracted, as shown in figure S4. During cell migration, the traction force in the leading region was the largest compared with the middle or trailing region, as well as in better alignment with the migration direction.

Traction Force Monitored During Guided Cell Migration

MC3T3-E1 cells were cultured on platforms with microposts as force sensors with integrated gratings as the guiding pattern. As shown in Fig. 6, the lamellipodia in the leading region was often in contact with the guiding pattern, whereas the trailing region was in contact with the sensing posts. Therefore, the traction force in the leading region could not be measured and only the traction force in the trailing region was analyzed. The number of cell covered microposts in the trailing region during guided cell migration was similar to cell migration without guidance, as shown in figure S5. The traction force at 0 min in the trailing region was small and randomly distributed, as shown in Fig. 6a. A time-lapse movie of a cell moving forward next to the grating pattern was shown in movie S2. During guided cell migration, the cell started to elongate and the leading region extended along the guiding gratings. The cell traction force in the trailing region also increased as shown in Fig. 6b. Cell traction force

FIGURE 6. (a)–(e) Force mappings during forward cell migration with guiding patterns. From 0 to 34 min, cell elongated from contracted shape followed by release of trailing region. Cell continued to migrate forward in second migration cycle from 34 to 67 min. (f)–(j) Force mappings during reversed cell migration with guiding patterns. From 0 to 36 min, cell elongated followed by cellular release on right side. From 36 to 58 min, cell reversed and migrated from left to right.

dropped after the trailing region was released and shifted towards the leading region when the next migration cycle began at 34 min, as indicated in Fig. 6c. Similarly, Figs. 6c–6e show the cell migrated during the second cycle from 34 to 67 min. The traction force in the trailing region first increased during cell elongation and dropped after the trailing region was released. This cell movement guided by the grating pattern was consistent with the previous work showing adherent cells stretched and migrated along the grating patterns.⁴²

As cells migrated along the guiding pattern, the changes in the normalized traction force in the trailing region and the cell speed were analyzed as a function of time, as indicated in Fig. 7a. Compared to cell migration without guidance, cell movement along the guiding pattern showed similar time-dependent traction force. Traction force in the trailing region first increased and then decreased after the release of the trailing region. The cell speed was low when the cell elongated as it hardly moved. The cell speed increased substantially after the retraction of the trailing region. These results indicated that cells underwent similar

FIGURE 7. (a) Normalized cell traction force in trailing region and speed as function of time of typical MC3T3 cell underwent forward migration with guiding pattern. Arrows indicate moments when trailing region was released, which were at 29 and 59 min, respectively. Durations of traction force to buildup and drop are indicated as t_i and t_D , respectively. (b) Normalized traction force in trailing region while cell shape contracted and elongated during migration with and without guiding pattern. (c) Durations of force to increase and decrease during migration with and without guiding pattern. Number of cells during guided and non-guided migration is 29 and 23, respectively.

migration cycles with the presence of the topographical clues. Hence, tracking the dynamic changes of the traction force in the trailing region is essential to understand the migration behavior of adherent cells.

The rate of traction force changes as a function of cell speed is shown in Fig. 8. During the period when the cells changed from contracted to elongated shape, the guided cells showed a higher rate of traction force increase compared to non-guided cells, as shown in Fig. 8a. Moreover, the high rate of traction force changes corresponds to higher cell migration speed as

FIGURE 8. (a) As cell shape changed from contracted to elongated, rate of cell traction force increase in trailing region as function of speed for cells with and without guidance. (b) During release of trailing region, rate of cell traction force decrease as function of speed with and without guidance.

cells moved faster on guided platform compared to without guiding pattern. During the release of the trailing region, the cell changed from elongated to contracted shape and the traction force dropped rapidly. The cell speed was higher than the period when the cell was originally elongated by comparing Figs. 8b to 8a. The cells also showed higher cell migration speed when the cells were guided by the parallel gratings during the period of release of trailing region as shown in Fig. 8b. These results showed that the measured traction force was lower during guided migration, perhaps due to less contact area with the substrate when guiding patterns were present. Hence, the lower traction force resulted in more rapid changes in traction force as well as higher cell migration speed for cells on platforms with guiding patterns.

Characteristics of Traction Force During Reversed Cell Migration

During cell migration, topographical guidance-induced direction changes could be observed, including a 180° direction reversal.^{42,47} Multiple factors have been reported to affect cell migration direction; however,

FIGURE 9. Normalized cell traction force in trailing region and speed as function of time during forward and reversed migration with guiding pattern. Arrows indicate moments when trailing region was released, which were at 21 and 53 min. Cell first migrated in forward direction, then reversed direction and continued in same direction.

the actual mechanism remains unclear. In this study, the dynamic changes in cell traction force during directional reversals were analyzed.

Reversed migration could be observed during guided cell migration, as shown in Figs. 6f–6j and the time-lapse movie S3. During the first migration cycle, cells moved forward from 0 to 36 min. The traction force increased in the trailing region as the cell elongated. The trailing region then released and retracted towards the leading region, as shown in Figs. 6f–6h. The cell then took a 180° direction reversal, as indicated in the second migration cycle from 36 to 58 min, when the new leading region developed in the opposite direction, as shown in Figs. 6h–6j.

As indicated in Fig. 9, cell traction force and speed during reversed migration on guiding pattern were measured. During cell elongation, the traction force in the trailing region increased and the cell speed was nearly constant. As the cell became elongated, the traction force in the trailing region continued to increase. At 21 min, the normalized traction force reached a maximum of 3.4 nN before the release of the trailing region. From 21 to 36 min, the traction force in the trailing region dropped rapidly to 0.8 nN. The trailing region retracted towards the leading region and the cell speed peaked at 6.8 μm/min. At this moment, the trailing region of the cell became the leading region from 36 to 53 min. At 53 min, the traction force peaked at 3.7 nN. From 53 to 58 min, the trailing region on the left hand side of the cell released and the 180° reversed cell migration was completed. The center of the cell shifted from left to right, and the cell speed peaked at 8.3 μm/min. The third cycle of cell migration went from 58 to 79 min as the cell continued to migrate forward to the right and the traction force continued to increase in the trailing region. During the three cycles of forward and reversed cell migration, the traction force showed similar behavior of the initial increase, followed by the fast release of the trailing region to

FIGURE 10. (a)–(c) Cell underwent forward and (d), (e) reversed migration without guiding pattern. (f) Normalized cell traction force and speed as function of time. Moments of trailing region release during migration are indicated by arrows at 48 and 108 min.

move forward or in a reversed direction with the rapid cell speed increase.

For comparison, the traction force was also measured during directional reversal without a guiding pattern, as shown in movie S4. Figures 10a–10e showed the micrographs of forward and reversed cell migration without guiding pattern. The normalized cell traction force and speed were analyzed as a function of time, as shown in Fig. 10f. From 0 to 48 min, the cell shape changed from contracted to elongated. The traction force in both the leading and trailing regions increased as the cell became elongated, with a larger traction force in the leading region than in the trailing region. At 48 min, the normalized traction force in the leading region was 4.1 nN compared with the 2.0 nN in the trailing region. From 48 to 54 min, the trailing region was released and the cell became contracted, and the speed peaked at 1.6 $\mu\text{m}/\text{min}$. After the original trailing region lost adhesions and the cell started to elongate during the second cycle from 54 to 108 min, the traction force started to increase in the original trailing region. The cell migration direction was reversed and the original trailing region became the new

leading region. At 108 min, the newly developed leading region showed a higher traction force of 6.2 nN compared with the 4.2 nN in the trailing region. The cell speed peaked at 3.0 $\mu\text{m}/\text{min}$ and the second cycle ended at 117 min with the release of the trailing region. These results show that directional reversal during cell migration is related to the inversion of the traction force in the leading and trailing regions of a cell. The region that has a higher traction force determines the cell migration direction.

CONCLUSIONS

Localized traction force significantly influences cell migration behavior, such as speed and direction. Topographical guidance can influence the dynamic development of traction force and can be used to regulate cell migration. Various cell traction force studies have been conducted on cell migration on flat surfaces. This is the first study cell traction force was monitor in real time, with and without guiding pattern, and in forward and reversed migration directions. The guiding pattern was used to control the cell migration speed and direction instead of using chemotaxis. While this work mainly studied cell traction force distributions under topotaxis controlled cell migration, cell traction force development with the addition of small molecule inhibitors will be carried out in future studies.

To monitor dynamic changes in cell traction force during migration, the traction force in the leading, middle, and trailing regions were tracked every minute. Cellular shape, traction force, and cell migration speed were analyzed continuously in repeated cycles as osteoblasts underwent directional migration. The cells switched between contracted and elongated shapes during each migration cycle. When cells started to migrate with contracted cell shapes, the traction force in the leading and trailing regions reached 5.8 ± 0.8 and 4.1 ± 0.4 nN, respectively. As both the leading and trailing regions protruded and the cell became elongated, the traction force in both regions increased. The traction force in the leading region became larger and better aligned with the migration direction than in the trailing region during cell elongation. After the traction force peaked at 7.1 ± 0.5 nN in the leading region and 6.0 ± 0.5 nN in the trailing region, the trailing region was rapidly released and the cell speed peaked. After release, the traction force dropped and the cell started the next cycle of migration. The traction force took 51.9 ± 6.7 min to build up while the cell remained elongated and 13.3 ± 1.8 min to drop after the trailing region released. The traction force in the middle region remained small at 2.7 ± 0.3 nN with little changes. The dynamic increments in traction

force in both the leading and trailing regions during cell elongation confirmed that cell spreading was initiated by the traction force, which is consistent with previous studies which showed that initiation¹⁹ and maturation^{14,41} of FAs are triggered by the increased traction force. The highly aligned net traction force in the leading region with the migration direction could be related to the development of microtubules, which tend to align with the migration direction and grow towards the leading region.¹⁵ As cells constantly changed their area and shape during directional migration, the cell traction force in the leading, middle, and trailing regions kept fluctuating. The cell shape continued to go through cycles of elongation and contraction as the cell moved forward. Under this dynamic movement, the net traction force equilibrium would not be reached until the cell stopped migration. Hence the fluctuation of the dynamic cell traction force is related to the variations of the cell area, shape, migration speed, and migration direction.¹¹ To have a better understanding of the dynamic changes in traction force during cell migration, further experiments will be performed to monitor the development of FAs, microtubules, and actin filaments.

Traction force was also analyzed when the cells underwent 180° direction reversal. The traction force in the trailing region was lower than the leading region during the first cycle of forward migration. After the cell reversed its migration direction, the traction force in the original trailing region became larger and it became the new leading region. Consistent with previous observations on cell migration under durotaxis, these results indicate that cells prefer to migrate towards the region with higher traction force.^{3,31} Previous studies showed that traction force in the leading region was propulsive to move the cell forward, whereas the traction force in the trailing region was resistive.^{2,9,37} The results shown in this study suggest that the region with the larger traction force is the leading region which determines the cell migration direction.

To compare guided and non-guided cell migration, parallel gratings were used to provide topographical guidance during cell migration. During guided cell migration, similar dynamic changes in traction force and cell shape were observed. Osteoblasts took 28.1 ± 3.3 min to build up traction force to 4.8 ± 0.3 nN in the trailing region as the cell became elongated. The traction force decreased rapidly, in 5.1 ± 0.4 min, in the trailing region to 2.2 ± 0.2 nN as the trailing region released and the cell contracted. Although the cyclic changes in cell traction force and shape were similar for guided cell migration, the magnitude of the measured traction force was lower and the migration speed was higher. During cell elongation, traction

force increased in the trailing region. This may be related to the cell–ECM interactions to form the elongated cellular shape for forward cell migration.^{5,20} In contrast to non-guided migration, the rates of increasing and decreasing traction force were higher for guided cell migration. The higher rate for traction force to build up during cell elongation along guided pattern was in agreement with the previous study showing that contact guiding introduced a more rapid protrusion of cellular filapodia on groove patterns.⁴² Previous work has shown that the disassembly of trailing FAs was due to the tensions from stress fibers,^{5,24} and this may be related to the faster traction force change rate during the release of the trailing region under guided cell migration.

The dynamic development of the cell traction force on micropost platforms with and without guiding gratings was comparable to the previously reported amoeboid cells under chemotaxis.^{1,8,36} It was reported that the cell traction force was higher near the two edges of a cell, which is in agreement with our study. The periodic buildup and reduction of the cell traction force were observed as cells migrated continuously with protruded leading regions and retracted trailing regions.^{1,8} Moreover, the higher cyclic change rate of traction force during guided cell migration resulted in faster migration was similar to previously reported results of higher cell motility for cells with shorter period of strain energy changes.⁸ During cell migration guided by parallel gratings, the cell migration speed was higher compared to non-guided migration, and lower traction force was observed in the trailing region compared to the leading region. These are new results that have not been reported previously since none of the previous studies involved guided cell migration. As topotaxis formed by the guiding pattern could be found in ECM and it could affect the cell polarization, and hence the distributions of the cell traction force from the leading to trailing regions. Therefore, the analysis of the cell traction force for the non-guided and guided cell migration is essential.

In summary, real time monitoring of cell traction force was carried out for cell migration in forward and reversed directions. Dynamic traction force development under guided, directional cell migration was studied for the first time. The results showed that cell–ECM interactions are regulated by topographical guidance that could influence cell migration speed and direction. These information on how cell traction force developed during cell migration on various surface topographies will be essential to obtain a better understanding of osteoblastic cell migration mechanisms and to provide control of cell movement for screening and diagnostics.

ELECTRONIC SUPPLEMENTARY MATERIAL

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CONFLICT OF INTEREST

J. Hui and S. W. Pang declare that there is no conflict of interest to report.

ETHICAL APPROVAL

No human studies were carried out by the authors for this article. No animal studies were carried out by the authors for this article.

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